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Prevalence of 16S rRNA methylase genes in Gram negative isolates in Athens Metropolitan area in a six month period

Konstantina Nafplioti[1], Irene Galani*[2], Helen Moraitou[3], Panagiota Giannopoulou[4], Paraskevi Chra[5], Maria Damala[6], Evangelos Vogiatzakis[3], Eleftheria Trikka-Graphakos[7], Vasiliki Baka[5], Eleni Prifti[6], Maria Souli[8]

[1]*National and Kapodistrian University of Athens, School of Medicine; 4th Dept of Internal Medicine, Infectious Diseases Laboratory*

[2]*National and Kapodistrian University of Athens, Faculty of Medicine; 4th Dept Internal Medicine, Infectious Diseases Laboratory*

[3]*"sotiria" General and Chest Diseases Hospital; Department of Clinical Microbiology*

[4]*"thriasio" General Hospital of Elefsina; Microbiology Department*

[5]*Korgialenio Benakio Hellenic Red Cross Hospital; Microbiology Department*

[6]*"alexandra" General Hospital of Athens; Microbiology Department*

[7]*Thriasio General Hospital of Elefsis; Microbiology Department*

[8]*University of Athens, School of Medicine; Attikon General Hospital; 4th Dept of Internal Medicine*

Background: Methylation of 16S rRNA is an important mechanism of high level aminoglycoside resistance among Gram-negative pathogens. The aim of this study was to investigate the prevalence of 16S rRNA methylase genes in consecutively collected Gram-negative isolates in the first semester of 2016 in Athens, Greece.

Material/methods: Single-patient**,** Gram-negative clinical isolates resistant to both amikacin and gentamicin (n=174), were consecutively collected during a six month period (Jan- June 2016) in five tertiary-care hospitals in Athens. All isolates were sent to a central laboratory for MIC determination to

amikacin, gentamicin, tobramycin [collectively referred to as 4, 6-disubstituted aminoglycosides, (4, 6-A)], apramycin and neomycin with the broth dilution technique. Isolates with MICs ≥256 mg/L to 4, 6-A were examined for the presence of 16S rRNA methylase (RMT) genes (*armA, rmtB, rmtC, rmtA, rmtD* and *npmA*) by PCR. Carbapenemase production was confirmed by PCR in all RMT-positive isolates.

Results: *A.baumannii, P.stuartii, K.pneumoniae, P.aeruginosa and E.coli* resistant to amikacin and gentamicin were isolated at participating institutions at a rate of 67.8%, 55.1%, 10.3%, 10.1% and 0.4%, respectively. One hundred and eight *A. baumannii* isolates of 113 tested (95.6 %), were positive for *armA*. The vast majority of *armA*-bearing *A.baumannii* strains were OXA-23 producers (97.2%) while three isolates (all from the same hospital) were OXA-24 producers (2.8%). All *P.stuartii* (n=14) isolates were VIM-producers and harboured *rmtB.* Eleven of the 29 *K.pneumoniae* isolates (37.9%) harboured *rmtB* (n=10) or *armA* (n=1). All *rmtB*-positive isolates were KPC-producers, while the *armA*–positive isolate was an OXA-48-producer. None of the 17 *P.aeruginosa,* isolates was positive for an RMT gene although some were highly resistant to 4,6-A tested (MICs >=512 mg/L). One *E.coli* isolate harboured *rmtB* and was a KPC-producer. The overall prevalence of *armA*-positive *A.baumannii* isolates was 64.8% and of *rmtB*-positive *P.stuartii* isolates was 55.1%. *K.pneumoniae* harboring *rmtB* or *armA* was isolated in low prevalence of 3.9% while only one *rmtB*-positive *E.coli* isolate was found in a total of 576 isolates (0.2%).

Conclusions: RMT production is an emerging mechanism of resistance, capable to compromise the clinical efficacy of aminoglycosides. High prevalence of RMTs was observed among *A.baumannii* and *P.stuartii* strains isolated in participating hospitals in Athens. Since the previous surveillance study by our group in 2009 (Galani et al. *CMI* 2012), the prevalence of RMTs in *K.pneumoniae* has increased from 0.4 to 3.9% while no RMTs have been found in *P.aeruginosa*. All RMT-positive isolates were carbapenemase producers.